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Distinct target genes and effector processes appear to be critical for p53-activated responses to acute DNA damage versus p53-mediated tumour suppression

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Abstract

The p53 tumour suppressor is the most frequently mutated gene in human cancer. This transcription factor can be activated by diverse cellular stresses, including DNA damage and oncogene activation. Through transcriptional induction of appropriate target genes, p53 can stimulate activity in a broad range of effector pathways, most notably cell cycle arrest, cellular senescence and apoptotic cell death. Insensitivity to cell death-inducing signals and deregulated proliferation are two key hallmarks of cancer cells. Given that p53 inhibits proliferation and induces apoptosis, it was widely believed that these processes are the most critical ones for p53-mediated tumour suppression. However, this dogma has been challenged. In striking contrast to p53-deficient mice, which all develop tumours before 250 days of age, mutant mice in which expression of the p53 target genes that are critical for induction of cell cycle arrest and apoptosis is impaired or abrogated are not cancer-prone. This demonstrates that distinct effector processes are critical for the p53-mediated acute response to DNA damage versus p53-mediated tumour suppression. The discovery that cell cycle arrest, senescence and apoptosis are not essential for p53-mediated tumour suppression re-launches the search for the p53 target genes and effector processes that are critical to prevent tumour development, with coordination of DNA repair being a leading contender.

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The role of p53 in the acute response to DNA damage

The transcription factor p53 is activated in response to a wide range of cellular stresses, including DNA damage (such as that induced by anti-cancer therapeutics), aberrant growth signals (e.g. as a consequence of oncogene activation), hypoxia, reactive oxygen species (ROS) and nucleotide depletion [1]. The role of p53 in the cellular response to acute DNA damage is particularly well characterised. Although *p53* mRNA is expressed relatively constantly, under normal cellular conditions (i.e. in the absence of stress) p53 protein levels are generally very low; indeed in many cell types below the level of detection. This is mostly due to a negative feedback loop involving the E3-ubiquitin ligases, MDM2 and MDM4 [2-4]. They inhibit inappropriate p53 activity by binding to its N-terminal transactivation domain thereby facilitating ubiquitination and consequent proteasomal degradation of p53 [5-7]. Additional proteins, such as HAUSP and DAXX, have also been implicated in regulating p53 protein, either by deubiquiylating p53 (HAUSP) or by enhancing p53 ubiquitylation (DAXX) [8]. In response

to cellular stress p53 becomes stabilised and activated through a variety of mechanisms. While we will briefly discuss how this occurs in response to DNA damage, the post-translational modifications that stabilise and activate p53 have been discussed in greater detail by David Meek and colleagues in the last issue of BioDiscovery [9].

Maintenance of genomic integrity is critical for cell survival (and in the germline for propagation of the species). Cells have therefore evolved complex systems for the recognition and repair of DNA damage. Recognition of DNA lesions, such as double strand breaks (e.g. induced by γ-irradiation), replication stress induced by hyper-proliferation (particularly important in the context of cancer) or by stalling of replication forks during cell cycling, can lead to activation and recruitment of the kinases ATM, ATR, Chk1 and Chk2 to sites of damage. ATM and ATR are serine/threonine kinases that are directly activated in response to DNA damage and function to phosphorylate key substrates, including p53, MDM2 and the downstream kinases Chk1/Chk2 [10-13]. Phosphorylation of p53 at serine 15, 20 and threonine 18 (all amino acid residues in human p53) is thought to activate p53 by enhancing its interaction with various members of the transcriptional machinery. This phosphorylation also inhibits degradation of p53 by interfering with the p53-MDM2 interaction [10, 12, 14-19]. Phosphorylation of MDM2 (on Serine 395) by ATM also further inhibits its interaction with p53 [20, 21]. Moreover, ATM also indirectly promotes p53 accumulation through phosphorylation of Chk2, activating it to phosphorylate serine 20 on p53 (aa residue relates to human p53). This modification is believed to interfere with the p53-MDM2 interaction [22, 23]. Once stabilised, p53 can undergo a range of additional post-translational modifications, including acetylation, phosphorylation, neddylation and methylation, which are thought to influence the transcriptional activity of p53 [24]. p53 regulates gene transcription (either positively or negatively) by binding as a homo-tetramer in a sequence specific manner to the regulatory regions of ~600 target genes [24-26]. Transcriptional responses and effector pathways unleashed by p53 in response to DNA damage fall into three broad categories: (1) growth arrest, including cell cycle arrest and cellular senescence, (2) repair of damaged DNA and (3) removal of cells that have sustained irreparable genetic damage by apoptosis.

Cell cycle arrest and cellular senescence

In response to DNA damage p53 can induce arrest of cellular proliferation. This can serve two functions: allowing cells time to repair their damaged DNA and blocking damaged cells from proliferating and thereby propagating aberrant DNA changes. This growth arrest can be transient or, in the case of cellular senescence, permanent.

The best characterised mediator of p53-induced cell growth arrest is p21 (also called WAF1/CIP1). *p21* was originally identified as a p53-responsive gene in a human glioblastoma cell carrying an inducible wild-type (wt) p53 expression construct [27]. Notably, over-expression of p21 was shown to suppress the growth of glioblastoma, lung adenocarcinoma and colon cancer derived cell lines *in vitro*. p21 binds directly to, and potently inhibits, the cyclin/CDK1 and cyclin/CDK2 cyclin dependent kinase complexes (CDKs). This blocks CDK-mediated phosphorylation of Rb, a process that is required for the release of the transcription factor, E2F, from Rb and thus progression from the G1 to the S phase of the cell cycle [28-31]. Mouse embryonic fibroblasts (MEF) lacking p21 were found to be resistant to DNA damage-induced cell cycle arrest at the G1-S boundary. While γ-irradiated wt MEFs underwent G1-S boundary cell cycle arrest, p21-deficient MEFs failed to arrest and consequently initiated replication of their damaged DNA [32]. Loss of p21 also promoted resistance to DNA damage-induced cell cycle arrest *in vivo* with comparable numbers of cells in S-phase observed in the gastrointestinal crypts of both γ-irradiated and untreated $p21^{-/-}$ mice whereas γ-irradiation caused a substantial reduction in cycling cells in wt mice [33].

Interestingly, p21 has also been suggested to function as an inhibitor of DNA damage induced cell death. Loss of p21 was shown to enhance the sensitivity of HCT116 colon cancer cells to daunomycin [34]. However, compared to wt MEFs, $p21^{-/-}$ MEFs treated with γ-irradiation did not exhibit enhanced levels of apoptosis and no differences in the proportions of TUNEL positive cells (a marker of apoptosis) were observed between gastrointestinal crypts of γ-irradiated wt versus $p21^{-/-}$ mice [32, 33]. Furthermore over-expression or knock-down of p21 failed to impair or enhance, respectively, the rate of apoptosis in a range of cancer derived cell lines when they were treated with the p53-activating compound Nutlin3a (an inhibitor of MDM2) [35]. By stalling cell cycle progression at the G1-S checkpoint p21 may enhance the survival of only certain dividing cells that have sustained DNA damage by preventing them from replicating their damaged DNA, a potentially catastrophic process that may trigger apoptosis or another cell death process. Consistent with the notion that p21 may only affect death in dividing cells, loss of p21 did not protect quiescent (non-cycling) cells from DNA damage induced apoptosis, with wt and $p21^{-/-}$ thymocytes (which are all G1 phase in culture) undergoing comparable levels of apoptosis in response to γ–radiation [32].

Additional p53 target genes, induced in response to DNA damage, have been implicated in the control of cell cycle progression at the G1-S and also other checkpoints.

For example, MEFs and intestinal epithelial cells lacking the transmembrane tyrosine phosphatase, Ptprv, failed to arrest at the G1-S boundary after γ-irradiation [36]. GADD45a is induced by p53 in several cell types in response to γ- as well as UV-irradiation [37, 38] and lymphocytes from *Gadd45a*$^{-/-}$ mice exhibit defective G2-M cell cycle arrest post UV-irradiation or treatment with methyl methansulfonate (MMS) [39]. GADD45a promotes cell cycle arrest by inducing disassociation of cyclin B1/CDK1 complexes, which are essential for entry into mitosis [40, 41]. Another p53 target, 14-3-3-sigma, was shown to regulate G2-M cell cycle arrest by sequestering CDK1 in the cytoplasm thereby blocking cyclin B1/CDK1 complex formation [42, 43]. In conclusion, p53-induced expression of target genes, such as *p21*, *ptprv*, *GADD45a* and *14-3-3-sigma*, promotes reversible cell cycle arrest at the G1-S or G2-M checkpoints to provide cells that have sustained DNA damage time to repair these lesions.

In response to DNA damage cells can also undergo irreversible growth arrest, a process called cellular senescence. Cells undergoing senescence become irreversibly halted in the G1 phase of the cell cycle but remain metabolically active [8, 44]. The process of cell senescence is accompanied by various morphological changes and the acquisition of a characteristic gene expression profile, including upregulation of INK4A [45], ARF [46, 47], p53 [48], PML [49, 50], PAI-1 [51] and p21 [52-54]. In non-transformed cells, induction of senescence is associated with the cessation of their proliferative potential that is characterised and governed by the decrease in telomere length during consecutive rounds of cell division (a process called replicative senescence) [55, 56]. Cellular senescence can also be induced by DNA damage or activation of certain oncogenes [45, 57]. A role for p53 in the induction of cellular senescence was first demonstrated when it was observed that p53 deficient MEFs failed to undergo replicative senescence [48, 58, 59]. Furthermore, it was shown that low dose γ-radiation of normal diploid fibroblasts induced a long-term growth arrest state resembling senescence that was associated with upregulation of p21 [57]. Moreover, doxorubicin treatment was found to promote permanent growth arrest with acquisition of a "senescence-like phenotype" in HCT116 colon cancer cells [60]. Induction of senescence in these cells was substantially impaired by the loss of p53 or its direct target p21 [60]. Finally, it was reported that cyclophosphamide treatment promoted sustained growth arrest with characteristic features of cellular senescence in Myc-driven lymphomas that also over-expressed Bcl-2 (and were therefore protected from apoptosis) [61]. This growth arrest was found to be p53-dependent, as p53-deficient Eμ-*myc* tumours over-expressing Bcl-2 failed to arrest and progressed rapidly during such treatment [61]. Interestingly, there is evidence that different genes and processes may be required for replicative versus DNA damage-induced cellular senescence, as p21-deficient MEFs were only defective in the latter [62]. Accordingly, p53 induces expression of not only p21 but also several additional target genes that are implicated in the induction and/or maintenance of cellular senescence, in particular PAI-1 and PML [50, 63, 64].

Apoptosis

In certain cells, particularly hematopoietic ones and intestinal epithelial cells, DNA damage will preferentially induce apoptosis, a form of programmed cell suicide responsible for the removal of unwanted or damaged cells from multi-cellular organisms [65]. Apoptosis involves the ordered destruction of damaged cells (in the absence of inflammation) and is characterised by a range of biochemical and morphological changes, such as plasma membrane blebbing, cellular surface exposure of phosphatidylserine, nuclear condensation and fragmentation, DNA cleavage and phagocytosis of dead cells by neighbouring innate immune cells [66-68]. Cell demolition in apoptosis is mediated by caspases (aspartate-specific cysteine proteases) that cleave hundreds of cellular proteins, including vital structural components [69, 70].

A role for p53 in apoptosis signalling was first indicated in studies that found that enforced expression of wt p53 induced characteristic features of apoptotic cell death [71-74]. Importantly, subsequent studies showed that lymphoid cells (thymocytes, pre-B cells, mature B cells and T cells) from *p53*$^{-/-}$ mice were profoundly resistant to apoptosis triggered by γ-irradiation, treatment with etoposide or certain other DNA damage-inducing chemotherapeutic drugs, both *in vitro* and *in vivo* [75-77]. Further studies showed that this effect was not limited to haematopoietic cells given that intestinal stem cells lacking p53 also exhibited profound resistance to γ-irradiation [78]. This p53-mediated apoptosis was recognised to be induced via the intrinsic apoptotic pathway since it could be inhibited by over-expression of pro-survival Bcl-2 family members or loss of pro-apoptotic Bcl-2 family members, particularly the combined loss of the multi-BH domain pro-apoptotic Bak and Bax [77, 79-82].

The intrinsic (also called "mitochondrial", "Bcl-2 regulated" or "stress induced") apoptotic pathway can be activated by developmental cues or diverse cell stressors including cytokine deprivation or DNA damage. Life versus death decisions in this pathway are controlled by the Bcl-2 protein family, which can be divided into three functional sub-groups: the pro-survival Bcl-2 family members (Bcl-2, Bcl-XL, Bcl-W, Mcl-1 and A1), the pro-apoptotic BH3-only proteins (Bad, Bid, Bik, Bim, Bmf,

Hrk, Puma and Noxa) and the multi-BH domain pro-apoptotic Bcl-2 family members (Bak, Bax and possibly Bok) [83, 84]. Apoptotic stimuli cause transcriptional, post-transcriptional and/or post-translational activation of the pro-apoptotic BH3-only proteins. They bind to the pro-survival Bcl-2 family members, thereby unleashing and activating Bax/Bak, but some BH3-only proteins (e.g. Bim, Bid) are also thought to directly activate Bax and Bak [85-88]. Activated Bax and Bak form homo-oligomers that trigger mitochondrial outer membrane permeabilisation (MOMP) by a mechanism that remains poorly understood. MOMP constitutes the "point of no return" in apoptosis signalling and results in release of apoptogenic factors, such as cytochrome c and Smac/DIABLO. This unleashes the cascade of caspase (aspartate-specific cysteine protease) activation that causes cleavage of hundreds of cellular proteins, thereby precipitating cellular demolition [67].

A direct link between p53 and the intrinsic apoptotic pathways was identified when it was found that the pro-apoptotic Bcl-2 family members Puma, Noxa and Bax are direct transcriptional targets of p53. The *puma* gene has a conserved p53 binding site (consensus sequence) within its first intron, and mutation of this site was shown to abrogate p53-dependent expression of Puma [89-91]. Studies with gene-targeted mice revealed that loss of Puma rendered thymocytes and many other cell types profoundly resistant to a range of p53-dependent apoptotic stimuli, including γ-irradiation and treatment with etoposide [92-95]. Interestingly, loss of Puma also rendered lymphoid cells (and several other cell types) resistant to certain death stimuli (e.g. cytokine deprivation, glucocorticoids, phorbol ester) [92-94] that activate the intrinsic apoptotic pathway [79] in a p53-independent manner [77].

Noxa, another BH3-only protein, is also a direct p53 target [96]. In response to γ-irradiation, thymocytes were found to induce Noxa expression in a p53-dependent manner [96] and siRNA-mediated knock-down of Noxa inhibited γ-irradiation induced apoptosis in E1A-transformed MEFs [97]. Experiments with gene-targeted mice showed that MEFs lacking Noxa were resistant to etoposide [93, 98] and UV-irradiation [99]. Interestingly however no protection against apoptosis induced by DNA damage was observed in lymphoid cells lacking Noxa [93]. These results suggest that Puma and Noxa may have functions in apoptosis that are cell type specific and/or specific to distinct forms of DNA damage. Studies with mice lacking both Puma and Noxa revealed that these two BH3-only proteins have overlapping functions in DNA damage-induced, p53-mediated apoptosis with *puma*$^{-/-}$*noxa*$^{-/-}$ thymocytes exhibiting protection from γ-irradiation *in vivo* comparable to *p53*$^{-/-}$ cells and more extensive than that of their *puma*$^{-/-}$ counterparts [100]. Collectively, these results demonstrate that Puma and Noxa account for all of the pro-apoptotic activity of p53 that is triggered by DNA damage. Puma is the major effector of p53-mediated apoptosis, whereas Noxa plays a more ancillary role. This is consistent with the finding that Puma can bind with high affinity and therefore neutralise all pro-survival Bcl-2 family members, whereas Noxa interacts only with Mcl-1 and A1 [88, 101, 102]. Another reason why Puma may be a more potent killer than Noxa is provided by several studies, which showed that only the former is able to bind to and directly activate Bax [86, 87, 103, 104].

So, what are the roles of other p53 targets that have been implicated as effectors of apoptosis? In the case of PERP, PIG3 and certain others [105-107], there are so far no data from gene-targeted mice to inform on their functions. However, given that combined loss of Puma and Noxa renders many cell types as resistant to DNA damage induced apoptosis as loss of p53, we predict that they have no essential function in p53-mediated apoptosis, but may act in other processes activated by p53.

Several reports have indicated that p53, activated by DNA damage, kills cells through stimulation of the "death receptor" (also called "extrinsic") apoptotic pathway. In this pathway (which operates largely in parallel to the "intrinsic apoptotic" pathway [108]) members of the tumour necrosis factor receptor (TNF-R) family with a "death domain" initiate apoptosis through FADD/TRADD adaptor protein-mediated caspase-8 activation [109]. The death receptor genes, *Fas*, *DR4* and *DR5,* and some other genes implicated in the "death receptor" apoptotic pathway were reported to be transcriptionally upregulated by p53 [110-118]. However, an essential role for "death receptor"-induced apoptosis in p53-mediated cell killing has been convincingly dismissed by several studies. For example, thymocytes and other haematopoietic cell types from mice deficient for Fas or Fas ligand (*lpr* and *gld* mice, respectively) die normally in response to γ-irradiation, while, conversely, *p53*$^{-/-}$ thymocytes and hepatocytes are normally sensitive to Fas-induced killing [108, 119-121]. Moreover, cells from mice lacking caspase-8 or FADD, which are indispensable for apoptosis induced by all "death receptors", are killed normally when exposed to p53-dependent apoptotic stimuli, such as γ-irradiation and etoposide [119, 120, 122-127]. Perhaps p53-mediated transcriptional induction of "death receptors" serves to sensitise cells that have sustained DNA damage or hypoxia to TNF, Fas ligand and related death ligands in order to orchestrate appropriate overall tissue responses to these stress conditions.

Several studies have indicated that p53 may trigger apoptosis via a non-transcriptional mechanism. It was reported that in response to γ-irradiation and certain other stresses, p53 can shuttle to the outer mitochondrial

membrane where it directly interacts with members of the Bcl-2 protein family to cause MOMP and consequent activation of the caspase cascade. Curiously, p53 was shown to bind to anti-apoptotic Bcl-XL as well as pro-apoptotic Bax and Bak to facilitate MOMP [128-132]. The physiological relevance of this proposed process remains uncertain. Notably, early work has shown that γ-irradiation-induced apoptosis (which depends on p53, see above) requires *de novo* RNA synthesis, as treatment of cells with the RNA synthesis inhibitor, 5,6-dichloro-1-beta-d-ribofuranosylbenzimidazole, promoted complete resistance to apoptosis (evidenced by absence of DNA fragmentation) [133]. Supporting this idea, several groups utilising knock-in mutant strains of mice bearing mutations that impair p53's transactivation function ($p53^{Gln25Ser26}$, $p53^{QS}$ and $p53^{25,26,53,54}$), have shown that cells from these animals are as resistant to p53 dependent apoptotic stimuli (e.g. γ- or UV-irradiation) as cells that lack p53 completely (i.e. cells from $p53^{-/-}$ mice) [134-136]. Based on these results and the aforementioned findings that combined loss of the pro-apoptotic BH3-only proteins Puma and Noxa renders cells as resistant to p53-mediated apoptosis as loss of p53 itself [100], we believe that the proposed post-translational processes have little or no role in p53-induced apoptosis under physiological conditions.

In conclusion, in response to DNA damage and certain other stress conditions (e.g. hypoxia, ROS) the tumour suppressor p53 is activated through complex post-translational mechanisms and then transcriptionally upregulates target genes, which then mediate cellular responses, including cell cycle arrest, apoptosis and coordination of DNA repair.

However, while we have a strong understanding of how these effector processes are orchestrated, questions remain as to how cell fate is determined after p53 activation, namely what determines whether in response to DNA damage a cell will undergo growth arrest and continue to survive or die? The choice between life and death is likely to be modulated by a wide range of factors, including the type and strength of the stress applied (although to date this has been only poorly correlated to cellular fate), differences in the inducibility of initiators of apoptosis or cell senescence, influence of other (p53-independent) signalling pathways that are activated, differences in the basal expression of pro-survival proteins and perhaps also factors that function downstream to limit activity of the effector pathways, such as inhibition of *Puma* induction by Slug [137] or inhibition of the mTOR pathway by p53. Gaining a fuller understanding of the mechanisms that control such choices of cell fate could provide critical information for the future development of anti-cancer therapeutics that trigger these processes more selectively (e.g. to favour cell killing over cell growth arrest).

The role of p53 in tumour suppression

Although originally identified as a proto-oncogene, it was soon revealed that p53 exhibited tumour suppressive actions. In particular, p53 was shown to inhibit E1A and Ras-induced transformation of rat fibroblasts [138]. This finding was strengthened by the discovery that the p53 gene was the most common target for genetic alterations in cancer, with ~50% of human cancers bearing mutations in this tumour suppressor gene. Importantly, many of the remainder harbour mutations or epigenetic changes that impair activation of p53 or some of its effector functions [1, 139, 140]. In humans, germline heterozygous loss-of-function mutations in p53 cause familial Li-Fraumeni syndrome, in which affected individuals are highly predisposed to developing various types of cancers (e.g. breast cancer, sarcoma and lymphoid malignancies) often at a young age [141-143]. Accordingly, mice lacking p53 [144, 145] or bearing loss of function point mutations in p53, (corresponding to amino acid residues in p53 that are altered in Li Fraumeni syndrome patients or in sporadic human cancers [146]), develop thymic lymphoma or sarcoma with 100% incidence between 150-250 days of age.

While the function of p53 in mediating cellular responses after acute and extensive DNA damage (e.g. in response to γ-irradiation or treatment with etoposide) is firmly established, the manner in which p53 suppresses the development of cancer is less well understood. Stabilisation and activation of p53 is known to occur in response to expression of certain onco-proteins, such as c-Myc or mutant Ras, which trigger transcription of the tumour suppressors $p14^{ARF}$ (humans) or $p19^{ARF}$ (mouse) [147]. $p14/p19^{ARF}$ functions to antagonise the p53-MDM2 interaction, thereby facilitating p53 accumulation, transcriptional activity and hence effector pathway activation [148, 149, 150]. Genomic instability is a hallmark of cancer, although whether this follows or precedes transformation is not always clear, and so it has been suggested that tumour suppression by p53 may also be activated through the DNA damage response pathway. Studies on human pre-cancerous lesions (e.g. non-small cell lung carcinoma, urinary bladder cancer, colorectal carcinoma, breast cancer, melanoma) have shown that they exhibit hallmarks associated with DNA damage pathway activation, including phosphorylation of the kinases ATM and Chk2, phosphorylation of histone H2AX and p53 accumulation [151, 152]. Interestingly, progression to malignant carcinoma (e.g. in non-small cell lung carcinoma and urinary bladder cancer) correlated with frequent mutation or loss of p53 [151, 152]. Over-expression of oncogenes that deregulate DNA replication, such as cyclin E, Cdc25A, E2F1, in U2OS osteosarcoma cells was shown to promote DNA damage

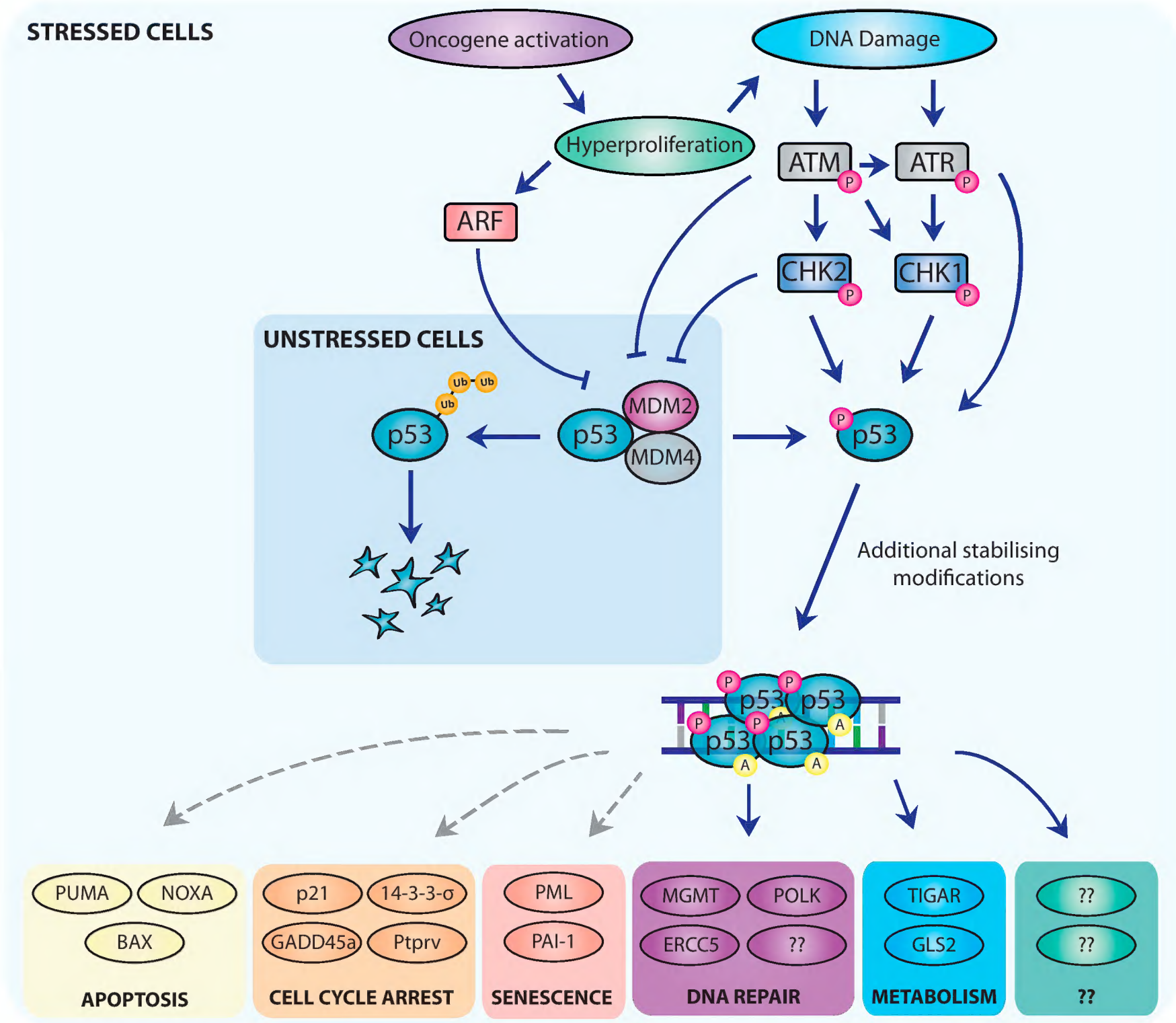

Figure 1. p53-mediated tumour suppression. The transcription factor p53 imposes a critical barrier against tumourigenesis. Almost all human cancers bear genetic alterations that either, directly or indirectly, render p53 non-functional and mice lacking p53 are prone to spontaneous tumour formation. The activity of p53 can be stimulated by the activation of certain oncogenes, such as deregulated expression of c-Myc or mutant Ras. This oncogenic stress promotes transcription of ARF, which then induces p53 stabilisation and accumulation by antagonising the p53-MDM2 interaction. Crosstalk between DNA damage-activated and oncogene-induced p53 stabilisation can occur when hyper-proliferation-induced replicative stress promotes DNA damage and hence activation of the ATM and ATR kinases. Phosphorylation of p53 and MDM2 by ATM/ATR inhibits p53 degradation by interfering with the p53-MDM2 interaction. Stabilised p53 undergoes further post-translational modifications, such as acetylation and neddylation, to become transcriptionally active. Binding as a homo-tetramer to specific DNA sequences, p53 mediates induction (and for some targets repression) of a wide range of specific target genes, which orchestrate activation of distinct effector processes. However, while apoptosis, cell cycle arrest and cellular senescence are critical effector processes induced by p53 in response to acute DNA damage, several studies have shown that these processes are largely dispensable for p53-mediated suppression of spontaneous tumour development. Instead the ability of p53 to modulate under-appreciated processes, such as DNA repair or control of metabolism, or perhaps control of presently unknown processes may be critical for p53-mediated tumour suppression.

response activation (evidenced by p53 and γH2AX phosphorylation) suggesting that cross-talk must exist between oncogene and DNA damage-induced pathways for p53 activation [151, 152].

Given the critical roles of apoptosis, G1-S boundary cell cycle arrest and senescence in the p53-regulated responses to acute DNA damage in non-transformed cells, it has been widely proposed that these same processes must also be essential for p53-mediated tumour suppression [26, 140, 153]. According to this hypothesis, activation of oncogenes (e.g. deregulated expression of c-Myc) will cause stabilisation of p53 via ARF-mediated inhibition of MDM2 whereas DNA damage, including that associated with early oncogenic events (e.g. DNA breaks in chromosomal translocations), will cause p53 activation via stimulation of the ATM/Chk2 kinases. Such activation of p53 in nascent neoplastic cells undergoing transformation would result in activation of apoptosis, cell cycle arrest or cellular senescence, thereby suppressing further progression towards malignant disease.

Interesting insight into the impact of restoration of p53 function in established malignant tumours that had developed as a result of loss or mutation of p53 came from studies of Gerard Evan and colleagues. They generated p53 knock-in mice in which the coding sequences of wt *Trp53* were fused to the coding region of a tamoxifen-sensitive version of the oestrogen oestrogen receptor ($p53ER^{TAM}$ mice). Expression of p53 in these mice could therefore be switched on or off in vivo by the administration of 4-hydroxy-tamoxifen (4-OHT) [154]. These mice were inter-crossed with the lymphoma-prone Eμ-*myc* transgenic mouse model, in which *MYC* expression is subjugated to the Ig heavy chain gene enhancer [155]. This model mimics the t[8;14] *c-Myc/IgH* chromosomal translocation that causes Burkitt's lymphoma in humans. The resulting Eμ-*myc*;$p53^{TAM/+}$ mice all developed lymphomas that showed deletion or loss of function mutations of the wt p53 allele [156]. These lymphomas were transplanted into recipients to investigate the effects of p53 restoration by addition of 4-OHT. Interestingly, even relatively short-term reactivation of p53 allowed enhanced survival of lymphoma-bearing mice. Lymphomas analysed 6 h after 4-OHT treatment exhibited markers of apoptosis (e.g. increased TUNEL staining, Annexin-V^+/Propidium $iodide^+$ and sub-G1 cells) [156]. These results suggest that restoration of wt p53 function suppresses sustained expansion of tumours (at least in the context of *Myc*-driven lymphoma) primarily through induction of apoptosis. The importance for apoptosis in p53-mediated (and p53-independent) suppression of lymphoma development in Eμ-*myc* mice was further demonstrated when it was shown that over-expression of Bcl-2 [157] or loss of the pro-apoptotic BH3-only proteins Puma and Noxa [158, 159] (direct p53 targets and essential effectors of p53-mediated apoptosis) and also loss of the BH3-only protein Bim [160] (not a p53 target) substantially accelerated lymphomagenesis in Eμ-*myc* mice.

In another study, Jacks and colleagues [161] developed knock-in mice in which expression of the endogenous p53 allele was placed under the control of a transcription-translation stop cassette flanked by *LoxP* sites. These so-called $p53^{LSL}$ mice were inter-crossed with Rosa26CreER mice, which express a tamoxifen regulated Cre recombinase in all cell types. The resulting $p53^{LSL}$;$CreER^{T2}$ mice are p53-deficient in the absence of tamoxifen. Accordingly, similar to the $p53^{-/-}$ mice, the $p53^{LSL}$;$CreER^{T2}$ mice succumbed to spontaneously forming thymic lymphomas and (more rarely) sarcomas between 120-250 days of age. Addition of tamoxifen to such tumour burdened animals promoted activation of the $CreER^{T2}$ recombinase, leading to excision of the transcription stop elements at *LoxP* sites with consequent expression of endogenous p53 protein in the tumour (and all other) cells [161]. This restoration of p53 resulted in the regression of both thymic lymphomas and sarcomas. However, while thymic lymphomas displayed widespread apoptosis in response to p53 restoration, surprisingly, sarcomas regressed in the absence of overwhelming apoptosis [161]. Instead, regressing sarcomas exhibited markers of senescence, such as β-galactosidase staining as well as expression of p15-Ink4b, p16-Ink4a, DcR2 and Dec-1 [161]. These observations suggest that the effector processes that p53 utilises for tumour growth suppression (in the context of established tumours) can vary in a cancer type-specific manner. Interestingly, since tumours were found to express high levels of the p53 activator $p19^{ARF}$ (a potent inhibitor of MDM2-mediated p53 degradation) and since restoration of p53 in normal tissues did not provoke widespread apoptosis or senescence, it was suggested that $p19^{ARF}$ (probably induced by the oncogenic lesions) was critical to prime tumour cells to respond to p53 restoration [161]. Further support for the notion that cellular senescence can play an important role in p53-mediated suppression of the expansion of malignant tumours came from a study performed by Lowe and colleagues. They explored the effects of p53 restoration in a mutant HRasV12-driven murine model of liver carcinoma, in which expression of p53 was reversibly knocked down using RNA interference [162]. As in the study from Tyler Jacks' group, restoration of p53 expression promoted regression of these carcinomas in mice in the absence of widespread apoptosis; instead regressing carcinomas exhibited markers of decreased proliferation and increased senescence [162].

These studies provide evidence that apoptosis and/or senescence play a critical role in p53-mediated regression of established tumours, and were widely thought to provide insight into the mechanism by which p53 suppresses the initiation of tumour formation. Unexpectedly, however, mice lacking single or multiple target genes that are essential for p53-mediated induction of apoptosis, cell cycle arrest or senescence, such as $p21^{-/-}$, $puma^{-/-}$, $noxa^{-/-}$ and $puma^{-/-}noxa^{-/-}$ mice, do not develop tumours (unlike the p53-deficient or p53 mutant mice) [32, 92-94, 98, 163]. Hence these studies, and several additional ones, challenge the essential nature of these three cellular processes for p53-mediated tumour suppression.

Evans and colleagues, utilising the aforementioned $p53ER^{TAM}$ knock-in mice probed the requirement for the acute DNA damage response triggered by p53 in the suppression of tumour development [164]. In the absence of 4-OHT, $p53ER^{TAM}$ mice are functionally p53-deficient and rapidly succumb to thymic lymphoma after exposure to low dose (2.5 Gy) γ-irradiation [164]. Cohorts of $p53ER^{TAM}$ mice were either treated with 4-OHT 6 days prior to γ-irradiation ("concurrent restoration of p53 function") or eight days after γ-irradiation ("delayed restoration"), a time point when the bulk of the acute

p53-mediated response, including large scale apoptosis of thymocytes, had been resolved [164]. Surprisingly, while "delayed restoration" of p53 promoted a significant (albeit relatively small) increase in tumour-free survival, "concurrent restoration" of p53 provided no survival advantage when compared to control irradiated mice ("no restoration of p53") [164]. Further studies using this mouse model revealed that the survival advantage observed in the "delayed restoration" cohort was entirely dependent on the expression of $p19^{ARF}$, which functions to stabilise p53 in response to oncogenic stress. This was based on the observation that *p53ER*^{TAM} mice lacking $p19^{ARF}$ did not exhibit enhanced survival post "delayed restoration" of p53. Taken together, these results suggest that the acute response to DNA damage and the pathology associated with widespread p53-activation, including widespread apoptosis of lymphoid cells, is dispensable for p53-mediated tumour suppression [164]. Moreover, the requirement for $p19^{ARF}$ for enhanced lymphoma-free survival suggested that tumour suppression, at least within this context, involves $p19^{ARF}$-mediated activation of p53 in rare clones of cells with activated oncogenes [164]. While this study constituted a considerable advance in understanding of how p53 suppresses tumour development, there is one caveat to this work. Mice homozygous for the *p53ER*^{TAM} allele are not functionally p53-null, as demonstrated by the failure to rescue the embryonic lethality induced by MDM2-deletion [165]. Therefore, residual expression of p53 over time could be sufficient to kill nascent neoplastic cells accounting for the observed survival advantage of these mice. An essential role for apoptosis in p53-mediated tumour suppression was further questioned when it was reported that mice deficient for endogenous (mouse) *p53* but carrying a human *p53* transgene, (*p53*^{SWAP} mice), were protected from rapid tumour development, although their cells were unable to undergo p53-mediated apoptosis in response to DNA damage [166].

In order to dissect the specific requirements for the different transactivation domains in p53 for tumour suppression, Attardi and colleagues generated a panel of *p53* conditional knock-in mice bearing mutations in specific residues within these regions [167]. Three mutants bearing alterations in either the first (L25Q, W26S: *p53*^{25,26} or *p53*^{QS} [168]), second (F53Q, F54S: *p53*^{53,54}) or both (L25Q, W26S, F53Q, F54S: *p53*^{25,26,53,54}) transactivation domains of p53 were utilised in this study, with regulatable expression of mutant p53 proteins facilitated by the introduction of a *LoxP-Stop-LoxP*-cassette within the first intron of the *p53* constructs. Microarray analysis on mutant HrasV12-transformed MEFs (transduced with a Cre recombinase) revealed that in response to DNA damage, mutation of both transactivation domains produced a "transactivation dead" phenotype, with similar gene expression profiles observed between *p53*$^{-/-}$ and *p53*^{25,26,53,54} homozygous mutant cells [167]. Conversely, mutation of the second transactivation domain alone appeared to have minimal impact on the transcription of the bulk of the p53-dependent target genes, with HRasV12-transformed MEFs from *p53*^{53,54} mice exhibiting a similar expression profile after DNA damage as those from wt (*p53*$^{+/+}$) cells. In contrast, mice with mutations in the first transactivation domain (*p53*^{25,26}) displayed impaired transcriptional induction of a large subset of p53 target genes after DNA damage, interestingly including the canonical targets *p21*, *Puma* and *Noxa* [167]. As a result of impaired induction of these critical effector genes diverse cells from these mice were markedly resistant to DNA damage-induced cell cycle arrest and apoptosis [167]. Remarkably, despite impaired induction of these critical effector processes *p53*^{25,26} homozygous mutant animals, in contrast to p53-deficient mice, did not exhibit accelerated tumour formation in a mutant Ras-driven transgenic model of non small cell lung cancer (NSCLC) with tumour burden observed comparable to wt (*p53*$^{+/+}$) controls [167]. Likewise, tumourigenesis induced by several other oncogenic drivers, such as induction of medulloblastoma caused by loss of one allele of *Ptch*, was considerably reduced in *p53*^{25,26} homozygous mutant mice compared to *p53*$^{-/-}$ animals [169]. Further analysis of the *p53*^{25,26} homozygous mutant mice indicated that in addition to retaining the ability to suppress tumour formation their cells remained able to efficiently undergo cellular senescence despite impaired induction of some (i.e. *p21* and *PAI-1*) but not all (i.e. *PML*) p53 target genes implicated in this process [167]. Accordingly, the authors proposed that cellular senescence (triggered by currently "under-appreciated" p53 target genes) may be critical for the tumour suppressive action of p53 [167]. In order to identify novel target genes involved in p53-mediated tumour suppression, Attardi and colleagues utilised microarray analysis to identify ~130 p53-regulated genes that upon DNA damage were differentially expressed in the *p53*^{25,26} homozygous mutant cells compared to their control ($p53^{-/-}$) counterparts. Further filtering of these results for genes known to be down-regulated in a variety of human and mouse cancers, resulted in a list of 14 candidate genes [167]. Of these candidates, some have been implicated in the repair of DNA damage (*Ercc5*, *Mgmt*, *Polk*), cytoskeletal function (*Arap2*, *Crip2*, *Def6*, *Kank3*), cell signalling (*Abhd4, Phlda3, Rgs12*) as well a range of other processes (*Sidt2*, *Ttc28*, *Ndrg4*, *Ctsf*) [167]. Knock-down of several of these target genes in HRasV12-transformed MEFs accelerated tumour growth *in vivo*, similar in extent to that afforded by knock-down of p53 [167]. These p53 target genes should therefore be considered as prime candidates for p53-mediated tumour suppression and it will be important to determine in which

cellular pathways they act.

Data from another *p53* mutant knock-in mouse strain however appears to rule out a critical role for cellular senescence in p53-mediated tumour suppression. In order to define the role for acetylation in modulating p53 function, Gu and colleagues generated a *p53* knock-in mouse strain bearing mutations in three conserved residues within the DNA binding domain of p53 (K117R, K161R and K162R: $p53^{3KR}$ mice) that are acetylated in response to DNA damage [170]. Acetylation of the equivalent residues in human p53 has been shown to play a critical role in controlling promoter-specific activation of p53 target genes and hence activation of the respective effector responses after cellular stress [8, 171]. In particular acetylation of K120 in human (K117 in mouse) p53 has been shown to be required for the induction of apoptosis, whereas loss of acetylation at K120 and K164 (K117 and K161 in mouse) was reported to abolish the ability of p53 to induce both apoptosis and cell cycle arrest [172]. Consistent with findings on human p53, cells from the $p53^{3KR/3KR}$ mutant mice were unable to undergo apoptosis or cell cycle arrest in response to DNA damage. These defects were shown to result from impaired transcriptional induction of *p21, Puma* and *Noxa*. Moreover, induction of several other classical p53 target genes was also impaired in cells from the $p53^{3KR/3KR}$ mutant mice, but they retained the ability to induce a small subset of p53 target genes, including *MDM2* and *TIGAR* [170]. Interestingly, fibroblasts from the $p53^{3KR/3KR}$ mutant mice also displayed impaired induction of replicative senescence. This defect was attributed to abnormally diminished induction of the p53 target genes *p21*, *PML* and *PAI-1* [170]. Remarkably, despite profound impairment in the induction of apoptosis, cell cycle arrest *and* senescence, unlike $p53^{-/-}$ mice, the $p53^{3KR/3KR}$ mutant mice were not tumour-prone, with only a low incidence (3/27) of late onset tumours observed [170]. Gene expression analysis showed that expression of the p53 target genes *TIGAR* and *GLS2*, which have been implicated in the control of metabolism [173], could still be induced efficiently in cells from the $p53^{3KR/3KR}$ mutant mice. Furthermore, in contrast to $p53^{-/-}$ MEFs, which exhibited elevated rates of glucose uptake, glycolysis and ROS production under normal culture conditions, MEFs from $p53^{3KR/3KR}$ mutant mice behaved like wt cells [170]. Collectively, these findings prompted the authors to suggest that regulation of metabolism but not induction of apoptosis, cell cycle arrest or cellular senescence is critical for p53-mediated tumour suppression.

Although these studies constitute a substantial advance in our knowledge of how p53 mediates tumour suppression, an important caveat of the studies of both of the aforementioned *p53* mutant mouse strains is that the expression of the critical effectors of apoptosis, cell cycle arrest and senescence (i.e. Puma and Noxa as well as p21, respectively) was only reduced but not abrogated. It therefore remained possible that residual p53-mediated expression of these target genes might be sufficient to suppress tumour development (although this was not sufficient to induce apoptosis, cell cycle arrest and senescence in response to acute DNA damage). In order to resolve this issue, we have recently generated mice that lack all of the critical effectors of p53-mediated apoptosis (Puma and Noxa) and G1-S cell cycle arrest as well as cellular senescence (p21) and investigated their predisposition to cancer. Consistent with previous studies of mice lacking Puma [93] or Puma and Noxa [100], thymocytes from $p21^{-/-}puma^{-/-}noxa^{-/-}$ were profoundly resistant to DNA damage-induced, p53-mediated apoptosis, in fact to an extent comparable to $p53^{-/-}$ cells. Furthermore activated, proliferating T lymphoblasts derived from $p21^{-/-}puma^{-/-}noxa^{-/-}$ mice failed to undergo G1-S boundary cell cycle arrest in response to γ-irradiation. Interestingly, while dermal fibroblasts from $p21^{-/-}puma^{-/-}noxa^{-/-}$ mice were profoundly impaired in the induction of DNA damage-induced cellular senescence when compared to fibroblasts from wt mice (>80% in wt versus ~30% in $p21^{-/-}puma^{-/-}noxa^{-/-}$ fibroblasts) they were less resistant compared to the $p53^{-/-}$ fibroblasts [174]. This finding can be explained as while p21 is important for induction of cellular senescence, it is not the sole p53-activated effector of this process with genes such as *PML* and *PAI-1* also being implicated [49,63, 64]. Remarkably, although induction of apoptosis and G1-S cell cycle arrest were completely abrogated and cellular senescence substantially impaired in these mice, none of the $p21^{-/-}puma^{-/-}noxa^{-/-}$ mice developed tumours (or any other disease) within the 500-day observation period. In contrast, all (n=125) $p53^{-/-}$ mice had succumbed to lymphoma or more rarely, sarcoma, between 120 to 250 days of age [174]. These results demonstrate beyond doubt that suppression of spontaneous tumourigenesis by p53 does not require p21-mediated cell cycle arrest and senescence (although not ruling out senescence induced by other effectors) as well as Puma/Noxa-mediated apoptosis.

These data do not, however, exclude that p53-induced apoptosis, cell cycle arrest and/or senescence may contribute to p53-mediated tumour suppression in the context of certain oncogenic driver mutations. Indeed, as discussed earlier, the loss of Puma or the combined loss of Puma and Noxa accelerated Myc-induced lymphoma development [158, 159]. It is, however, important to note that the loss of both Puma and Noxa accelerated lymphoma development in Eμ-*myc* mice to a considerably lesser extent than loss of even a single allele of p53 (median survival Eμ-*myc*;$p53^{+/-}$: <40 days versus Eμ-*myc*;$puma^{-/-}$;$noxa^{-/-}$: 66 days). This finding demonstrates that even in this context where induction of apoptosis (via

Puma/Noxa) does contribute to p53-mediated tumour suppression, additional processes activated by p53 must be even more critical. Collectively, these studies suggest that while apoptosis, cell cycle arrest and senescence are required for the p53-activated cellular responses to acute DNA damage, these processes are not essential for p53-mediated suppression of tumour development. It therefore appears that p53 functions as a multi-faceted transcription factor that utilises distinct transcriptional programs to respond to different cellular stresses, i.e. utilising primarily apoptosis, cell arrest and senescence to respond to acute DNA damage but necessitating distinct processes for tumour suppression.

So the question arises, which effector processes controlled by p53 are critical for tumour suppression? As mentioned above, in their studies with the *p53*$^{3KR/3KR}$ mice, Gu and colleagues concluded that coordination of metabolism might be critical [170]. The role of p53 in the control of metabolism and the contribution of this process to tumour suppression are fields of increasing interest. Under normal conditions where oxygen is not limiting, cells primarily derive their ATP from the conversion of glucose to pyruvate (glycolysis). Pyruvate is then shunted into the TCA cycle leading to the generation of NADH intermediates for oxidative phosphorylation (aerobic respiration) [175]. In contrast, cancer cells (particularly in hypoxic areas of solid tumours) are known to undergo a metabolic switch to utilise "aerobic glycolysis" as a primary source for ATP, in a process termed the "Warburg effect" [175]. In this process, pyruvate is primarily converted into lactate and intermediates formed during the TCA cycle are redirected to other biochemical processes that aid cell proliferation by providing essential metabolites, such as acetyl-CoA for fatty acids and ribose for nucleotides [175]. Interestingly, p53 was recently shown to be a transcriptional activator of genes that regulate metabolism, such as *GLS2* and *TIGAR*, which control glycolytic flux within cells [173, 176, 177]. Furthermore, p53 was shown to directly suppress expression of GLUT1 and GLUT4 and indirectly suppress GLUT 3, glucose transporters that regulate cellular glucose levels [178]. Therefore, loss of p53 function would facilitate a metabolic switch to aerobic glycolysis thereby promoting increased proliferation and survival of tumour cells. Another hallmark of deregulated metabolism is the increased production of intracellular reactive oxygen species (ROS). This can result in ROS-induced DNA damage, thereby enhancing the likelihood for accrual of DNA mutations that would promote neoplastic progression [179].

While coordination of metabolism represents a credible and interesting possibility to explain how p53 suppresses tumour formation, other known and possibly also some unknown p53 effector processes may be equally or even more critical. Interestingly, we found that upon γ-irradiation, cells from *p53*$^{-/-}$ animals (which are tumour-prone) displayed impaired induction of genes implicated in DNA repair and a trend towards abnormally increased persistence of γH2AX foci (a marker of detection of double-strand DNA breaks and initiation of their repair) [174]. In contrast, cells from *p21*$^{-/-}$*puma*$^{-/-}$*noxa*$^{-/-}$ mice (which are not tumour-prone) behaved like wt cells in these respects. Consequently, we hypothesise that proper coordination of DNA repair by p53 may be critical to suppress tumour development. Known as the "guardian of the genome" p53 was initially implicated in the coordination of DNA repair via its ability to induce cell cycle arrest post DNA damage, a process that would allow for sufficient time for DNA damage to be properly repaired [37, 180]. The connection between p53 and DNA repair has been substantially affirmed with the finding that p53 can transcriptionally upregulate a number of genes that have critical roles in DNA damage recognition and repair, including *p53R2*, *DDB2*, *DDIT4*, *ERCC5*, *FANCC*, *GADD45A*, *MGMT*, *MSH2*, *POLK*, *XPC* [37, 167, 181-187]. Moreover, p53 has also been suggested to facilitate DNA repair by directly interacting with certain DNA repair factors at sites of DNA lesions [188]. While the loss of individual p53 target genes implicated in DNA repair fails to reproduce the marked spontaneous tumour predisposition of p53-deficient mice, loss of several of these targets have been shown to accelerate tumourigenesis in radiation-induced models of carcinogenesis. For example, XPC-deficient mice are prone to UVB-induced skin cancer [189] and GADD45a-deficient mice are prone to γ-irradiation-induced carcinogenesis [190]. Importantly, mutation or loss of some of these genes in humans are linked to disorders in which individuals are prone to various forms of cancer. For example, *XPC* mutations in *Xeroderma pigmentosum* are associated with a high risk of skin cancer [191]; *MSH2* mutations cause hereditary non-polyposis colorectal cancer (HNPCC) [192, 193]; and mutations in *FANCC* lead to Fanconi anaemia, a disorder characterised by progressive bone marrow failure and marked predisposition to myeloid leukaemia and squamous cell carcinoma [194, 195]. Interestingly, cells undergoing early stages of neoplastic transformation often display signs of DNA damage response signalling, including ATM and Chk2 phosphorylation as well as p53 accumulation [151, 152]. This indicates that p53-mediated tumour suppression may involve recognition and repair of DNA lesions caused by replicative stress in cells undergoing oncogene-driven hyper-proliferation. According to this model, activation of p53 would trigger repair of such lesions and thereby minimise acquisition of cooperating mutations that would otherwise promote progression towards a malignant phenotype.

Another intriguing idea is that p53 may also function as a guardian of normal cellular stresses, in addition to

its defined roles in responses to acute stresses. In healthy cells p53 is constitutively expressed, albeit restrained at low levels. Accordingly, constitutive expression of p53 would allow for its transient activation in response to normal cellular stresses such as, DNA strand breaks that occur during replication or transient changes in cellular metabolites. Such transient p53 activation would be predicted to be insufficient to activate apoptosis or cellular senescence, either because these stresses induce different overall transcriptional programs or simply due to the transient nature of the p53 response in this context (e.g. to elicit apoptosis, Puma/Noxa levels must increase sufficiently to overcome the protective effects of the pro-survival Bcl-2 family members present within a given cell). Such transient p53 activation, although at low levels, might be sufficient to counter early tumourigenic events, such as oncogene activation.

Regardless, it is apparent that much remains unknown about how p53 mediates tumour suppression, and so, the search for the p53 target genes and the processes they regulate that are critical to protect us from developing cancer continues. Much work remains to validate the function of the newly discovered candidate genes implicated as critical for tumour suppression in the aforementioned studies (e.g. through use of gene-targeting strategies on targets identified by Attardi et al [167] and Gu et al [170]). Furthermore, there is a need to re-examine our current knowledge of p53 target genes to ensure it encompasses all actual p53 targets. Finally, understanding if (and how) the transcriptional programs initiated by p53 differ (perhaps subtly) in response to distinct cellular stressors (e.g acute DNA damage versus oncogene activation) may provide insight into p53's functions as a tumour suppressor. Understanding and harnessing newly implicated effector processes could lead to more efficacious anti-cancer treatment strategies, for example through development of novel cancer therapeutics that reactivate these processes within cancer cells or alternatively in pre-neoplastic cells of people bearing cancer-predisposing gene mutations. This may be pertinent to Li-Fraumeni patients with germline mutations in p53 or individuals bearing mutations in genes where the resulting tumours exhibit high rates of p53 mutation (e.g. women bearing BRCA1/BRCA2 mutations). Selective activation of effector processes critical for tumour suppression may also result in reduced treatment side effects. For example, interventions that do not elicit widespread apoptosis would minimise lymphoid and gastrointestinal tract depletion.

Conclusions

To conclude, p53 imposes a critical barrier against the development of cancer. However, the mechanisms by which p53 mediates tumour suppression remain elusive. Surprisingly, it appears that suppression of spontaneous tumour formation by p53 utilises distinct effector processes from those that are critical for the cellular responses to acute DNA damage. Experiments utilising *p53* mutant knock-in mice or gene-targeted knock-out mice lacking well-characterised p53 effector genes have demonstrated that induction of apoptosis, cell cycle arrest and cellular senescence, even in combination, appear largely dispensable for the ability of p53 to suppress spontaneous tumour development. Instead, p53 must mobilise currently underappreciated processes, such as coordination of DNA repair, control of metabolic adaptation or perhaps even currently unknown processes to suppress cancer formation. Defining the effector processes that are critical for p53-mediated tumour suppression and how the signalling pathways responsible for these processes are triggered by oncogenic events remain critical goals of future research. Detailed understanding of these processes will have great potential to aid in the development of novel strategies for cancer therapy and possibly even cancer prevention.

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